Bovine Anaplasmosis

Disease Monograph Series – 09

Parasite | Anaplasmataceae | *Anaplasma marginale* | Water Buffalo | Cattle

IDRC | Bartay

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Acronyms

AU	African Union
AU-IBAR	African Union Inter-African Bureau for Animal Resources
BA	Bovine anaplasmosis
CAT	Card agglutination test
CVO	Chief Veterinary Officer
DALY	Disability-adjusted life year
DIVA	Differentiate infected from vaccinated animals
DVS	Director Veterinary Services
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
IAEA	International Atomic Energy Agency of the United Nations
IM	Intramuscular
MSP	Major surface protein
NGO	Non-governmental organization
OIE	World Animal Health Organization
PCR	Polymerase chain reaction
SHF	Small holder farmer

TBD	Tick borne diseases
TPP	Target Product Profile
WHO	World Health Organization of the United Nations

Executive Summary

Etiology and relevance

Bovine Anaplasmosis (BA), an arthropod-borne disease of ruminants, is predominantly caused by *Anaplasma marginale*, an obligate intraerythrocytic parasite in the genus Anaplasma belonging to the family Anaplasmataceae of the order Rickettsiales. The organisms in this order were recently reclassified based on genetic analysis of 16S rRNA, groELS and surface protein genes. The genus Anaplasma includes three species that can infect ruminants, *A. marginale*, *A. centrale* and *A. ovis*. *A. marginale* and *A. centrale* infect cattle, but differ in morphology, virulence and geographical distribution.

A. marginale was discovered and first described in 1910 by Arnold Theiler in South Africa, who shortly after noted also a less pathogenic strain which he called *A.centrale*: this second discovery has had a tremendous impact in the control of bovine anaplasmosis as he could demonstrate its ability to protect inoculated cattle against *A. marginale* challenge. The vaccine he then developed is still used to date, with the same strain for bovine anaplasmosis.

Epidemiology and transmission

Anaplasmosis has a worldwide distribution particularly in tropical and subtropical regions; it is also seen in some temperate areas. The disease is endemic in Africa, South and Central America, southern Europe, the Far and Middle East, India, Russia, and Australia.

While *A. marginale* and bovine anaplasmosis affects cattle and water buffaloes in Asia, several wild ungulate species are also affected and play an important role in the disease epidemiology. *Bos indicus* cattle breeds appear to possess a greater resistance to *A. marginale* infection than *Bos taurus* breeds, but variation in resistance of individuals within breeds of both species may well occur.

Anaplasma species are transmitted either mechanically or biologically by arthropod vectors. In general, tick vectors of *A. marginale* include *Boophilus spp*., selected *Dermacentor spp*., *Ixodes ricinus* and *Rhipicephalus spp*., while *Amblyomma spp*. do not appear to transmit *A. marginale*. Reviews based on careful study of reported transmission experiments list up to 20 of these different ticks as capable of transmitting *A. marginale*. Intrastadial or transstadial transmission is the usual mode, even in the one-host Rhipicephalus species. Male ticks may be particularly important as vectors; they can become persistently infected and serve as a reservoir for infection.

Clinical signs

Due to the fact that *A. marginale* primarily infects erythrocytes, anaemia, jaundice and sudden death are characteristic signs of anaplasmosis. Other symptoms of bovine anaplasmosis include fever, lethargy, weight loss, lowered milk production, and abortion. It is often fatal in older cattle if they are not treated early

The severity of bovine anaplasmosis increases with age: calves under six months of age rarely become ill; between 6-12 months they usually develop mild disease, which is more acute at 1-2 years old; finally, adults over two years old suffer acute and often fatal disease.

Cattle that recover from acute disease usually remain persistently infected showing cyclical rises in rickettsemia, which trigger the generation of antigenic variants that escape immune control and multiply rapidly, before they stimulate a variant-specific immune response that brings the infection under control again. Due to this consistent stimulation, persistently infected cattle maintain a strong immune response against *A. marginale*, and are protected from disease when subsequently challenged with the homologous *A. marginale* strain though, sadly, they act as reservoirs of infection for naïve cattle.

Incidence / Prevalence and economic impact

Though known to be very prevalent in areas where the disease is endemic, very little prevalence data exist for certain regions. Bovine anaplasmosis has a serious economic impact on the cattle industry of endemic areas, and more specifically in the target countries, essentially due to animal mortality, morbidity and reduced productivity, cost of acaricides and other treatment, including chemotherapy and immunisation where applicable. In most countries economic costs directly associated to Anaplasmosis are included in overall costs of tick borne diseases. Only in Eastern and Southern Africa, the only regions of the African continent where data exist, there are more than 80 million animals that are at risk of Anaplasmosis with costs for countries like Tanzania estimated at 48 million USD annually. In India, Anaplasmosis is considered as one of the top 10 economically important diseases affecting ruminants.

Diagnostics

Microscopic examination of blood or organ smears is the most common method of identifying Anaplasma in clinically affected animals. Several serological tests are also employed in epidemiological studies: complement fixation (CF) test, capillary agglutination assay, card agglutination test (CAT), indirect fluorescent antibody (IFA) test, as well as various ELISA such as a cELISA, indirect ELISA and dot ELISA. The two serological tests currently preferred for identifying infected animals are the cELISA and the CAT. PCR have also been developed that are capable of detecting the presence of low-level infection in carrier cattle and tick vectors.

Control

The control of Bovine Anaplasmosis varies according to regions and their level of endemicity, with major components being the maintenance of *A. marginale*-free herd, vaccination, tick control, treatment with tetracycline during acute disease and administration of low-level tetracycline for prevention of clinical disease.

Tick control is practised in many parts of affected countries through use of acaricides or improved animal management. Acaricides have however been linked to several tick resistance cases, thus compromising ther use. Maintenance of a stable disease situation by strategically allowing natural exposure of calves to tick-borne diseases, including anaplasmosis, is often practised in Southern and Eastern Africa.

Treatment or chemotherapy with antibiotics is the most widely used control measure for Bovine anaplasmosis, with Tetracycline antibiotics being by far the predominant treatment method.

Vaccination is the control method used since the description of bovine anaplasmosis in 1911 in South Africa. Live vaccines based on the Theiler *A. centrale* strain are used in several countries; they yields partial protection against challenge with virulent *A. marginale*.

Vaccination and vaccination strategies

Even though inactivated vaccines have been used in the USA, and most of them discontinued since the 1990s, the live vaccine based on the *A. centrale* from Arnold Theiler in 1911 is still used to date in all countries using live vaccine. Such vaccine, now delivered in a frozen form, is used to date in South Africa, Israel, Latin American countries and Australia, where it forms part of a multivalent vaccine, and supplied chilled.

Both killed and live vaccines rely on erythrocyte-derived antigen sources. None of the two vaccine forms prevent cattle from becoming persistently infected with *A. marginale* or becoming reservoirs of infections.

The development of an effective bovine anaplasmosis vaccine preventing the infection is complicated by the increasing numbers of *A. marginale* field strains that occur in a given geographical area. While research carried out in the last two decades has contributed greatly to our knowledge of the antigenic composition of *A. marginale*, it has not, as yet, led the development of novel vaccines using molecular technologies.

Live blood Anaplasmosis vaccine has several disadvantages including: lack of cross protection with several strains of *A. marginale*, residual virulence especially when administered in adult animals, challenges linked with the cold chain as the current vaccines used in Africa are frozen and have to be maintained at temperature below 0°C during transportation, a condition difficult to meet in Africa. There is also the risk of transmitting other diseases through the blood.

Of the promising opportunities for new generation vaccines, is proteomic and genomic work at at the University of Washington (USA) that has permitted to identify new proteins within the outer membrane immunogen in addition to the well-characterized Major Surface Proteins (MSP1–MSP5), which hold promises for potential

vaccine; but they still have to be further selected and evaluated in cattle studies. Cell culture systems also, by propagating *A. marginale* in a continuous tick cell line derived from embryonic *Ixodes scapularis,* have also yielded less infective vaccine antigens. A group in The Pirbright Institute, (UK) has published this year a paper on their successful cultivation of *A. centrale* in tick cells. There will be a need to assess the vaccine characteristics of this cell derived *A. centrale* in challenge studies.

The future of Bovine Anaplasmosis vaccines and vaccination

Given the difficulties in producing and transporting the current live attenuated *A. centrale* vaccine, only South Africa produces and use the vaccination approach to the control of BA, and to a lesser extend Zambia. A number of approaches could be considered in order to improve the availability and use of vaccination in the control of BA.

The current live Theiler *A. centrale* vaccine has serious limitations that would prevent its use in more countries. With limited research and work toward the development of a vaccine over the past few years, and the lack of funds toward such research, a new generation vaccine may be a long way away. There may be a possibility of evaluating in target animal the candidates selected by the Washington State University group. These candidate vaccines are likely to lead to a potentially very efficacious and safe vaccine.

The “quick win” would be the use of the newly established cell culture system for *A.centrale* vaccine strain developed at The Pirbright Institute, which could allow the production of a less risky cell-based vaccine. Additionally, work on lyophilisation of the vaccine could be pursued. A cell-based lyophilised bovine anaplasmosis vaccine would then have a major impact on in the control of bovine anaplasmosis.

Clinical disease overview

Etiology

Bovine Anaplasmosis is an arthropod-borne disease of ruminants, first described by Arnold Theiler in South Africa first described the disease in 1910, which is caused predominantly by *Anaplasma marginale*, and is generally characterized by fever, progressive anaemia and icterus.

The pathogen is an obligated intraerythrocytic parasites in the genus Anaplasma belonging to the family Anaplasmataceae of the order Rickettsiales

Anaplasma species were originally regarded as protozoan parasites, but further research showed they had no significant attributes to justify this description. Further to comprehensive genetic analyses of the 16s rRNA, groESL and surface protein genes, the genus *Anaplasma* is now reclassified as one of four distinct genera in the family Anaplasmataceae, alongside *Ehrlichia, Wolbachia and Neorickettsia.* The organisms within this family are Gram-negative, obligate intracellular bacteria vectored primarily by ticks. A unique feature of these organisms is that they reside and replicate within parasitophorous vacuoles in the host cell cytoplasm of their tick and vertebrate hosts [19].

After the identification by *A. marginale* in 1910, Arnold Theiler identified and described a less pathogenic strain which he called *A.centrale* and which turned out to have a tremendous impact in the control of bovine anaplasmosis to this day. He based the distinction between *A. marginale* and *A. centrale* (Fig. 1) on:

- the different position taken up by the two parasites within the erythrocyte,
- the presence of slightly smaller sized organisms in *A. centrale* infections,
- the lower virulence of *A. centrale*, and
- the incomplete cross-immunity to *A. marginale* demonstrated in animals recovered from *A. centrale* infections. [32]

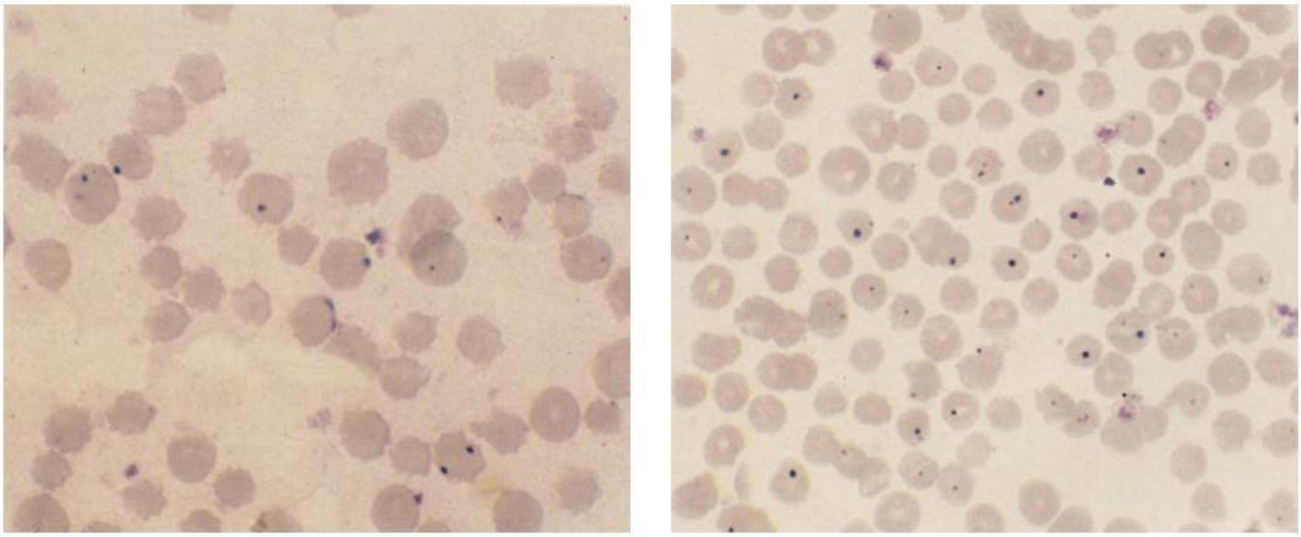


Figure 1: Blood smears of *A. marginale* (left) and *A. centrale (right).* [35]

While *A. marginale* is the main pathogen causing Bovine anaplasmosis, *Anaplasma centrale* is capable of producing a moderate degree of anaemia, but clinical outbreaks in the field are extremely rare. New species of Anaplasma, *A. phagocytophilum* and *A. bovis*, with a primary reservoir in rodents, have been reported to infect cattle, but do not cause clinical disease [20][35].

A. marginale has a small genome estimated at 1.2 to 1.6 Mb. There are six major surface proteins (MSPs) that have been identified on *A. marginale* derived from bovine erythrocytes and found to be conserved on tick- and cell culture-derived organisms [18]: MSP1a, MSP1b, MSP2, MSP3, MSP4 and MSP5. Three of these MSPs, namely MSP1a, MSP4 and MSP5, are from single genes and do not vary antigenically during the multiplication of the bacterium, while the other three, MSP1b, MSP2 and MSP3, are from multigene families and may vary antigenically, most notably in persistently infected cattle [21]. However, recent results demonstrated selection of MSP2 sequence variants in persistently infected ticks.

Due to the fact that MSPs are the most abundant, immunogenic and highly expressed, they have significance in vaccine research and have been part of several new generation vaccine studies. MSP1a, MSP1b and MSP2 have been shown to be adhesins involved in *A.marginale* interaction with cattle and ticks, with MSP1a having been shown to be the adhesin that binds erythrocytes, tick cell extracts and the gut cells of tick vectors [8]. MSP4 and MSP5 are both encoded by single-copy genes, and are highly conserved. As such they are useful for phylogenetic analysis [9] and diagnostics, with MSP5 used for a commercial diagnostic ELISA [43]

Epidemiology

Susceptible animal species

A. marginale and bovine anaplasmosis affects only ruminants, domestic and wild ruminants. In Asia the disease is a problem in cattle and water buffaloes.

It has long been known that Anaplasma spp. can infect certain wild ungulate species, but there is mounting evidence that a wide host range may be involved, e.g. the giraffe, African buffalo, eland, greater kudu, nyala (*T. angasii*), waterbuck, etc. Anaplasma infections are readily maintained in cattle populations. The importance, if any, of wild ungulates as a reservoir for the transmission of Anaplasma spp. to cattle is unknown. [35]

Breed differences in susceptibility

Several studies have been carried out to determine if there is a difference in susceptibility for *A. marginale* infection between local African and Asian breeds (*Bos indicus*), European breeds (*Bos taurus*) and their crosses. *B. indicus* have been shown to be susceptible to anaplasmosis and may also develop clinical symptoms. Under experimental inoculation *B. taurus*, *B. indicus* and their crosses were equally susceptible to *A. marginale* and developed similar responses in packed cell volume (PCV)-depression and maximum rickettsemia detected microscopically [6]. Bock et al. [6] showed that 50 % of *B. indicus* needed treatment to recover in contrast to 100 % of the pure *B. taurus*. When comparing *B. indicus* and crosses after artificial infection with *A. marginale* via *R. microplus* there were no significant breed differences.

Distribution

Anaplasmosis has a worldwide distribution particularly in tropical and subtropical regions, it also seen in some temperate areas. The disease is endemic in Africa including Egypt, South and Central America, southern Europe, the Far and Middle East, India, Russia, and Australia. See Figure 2.

Clinical anaplasmosis was first described in Indian cattle from the State of Odisha in 1963. Subsequently, *A. marginale* infection was detected in livestock of Uttar Pradesh, Punjab, Haryana, Tamil Nadu, Karnataka, Jammu and from parts of north and central India. In recent times, anaplasmosis has been recorded in Jammu, Karnataka, Haryana and Tamil Nadu [11].

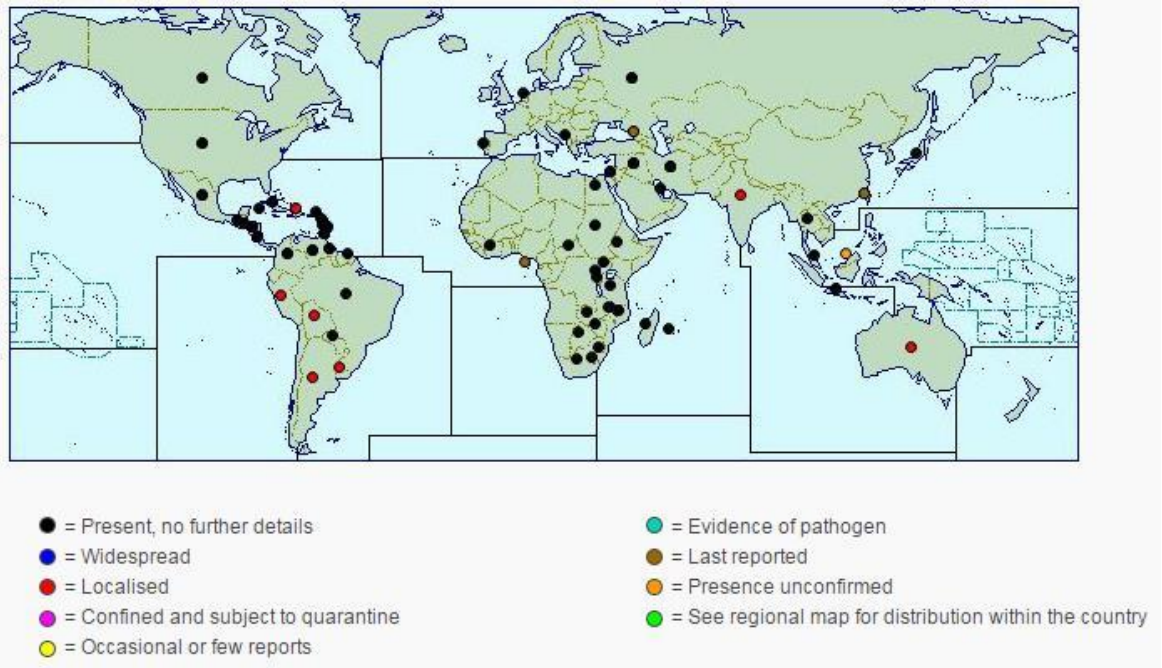


Figure 2: Global distribution of bovine anaplasmosis source: http://www.cabi.org/isc/datasheet/91734

Vectors of Bovine anaplasmosis and Transmission

A. marginale develops persistent infections in cattle and tick hosts. While erythrocytes appear to be the only site of infection in cattle, *A. marginale* undergoes a complex developmental cycle in ticks and transmission occurs via salivary glands during feeding. Many geographic isolates occur that vary in genotype, antigenic composition, morphology and infectivity for ticks.

Anaplasma species are transmitted either mechanically or biologically by arthropod vectors. Various tick and haematophagous fly species are vectors of *Anaplasma spp: Ixodid* ticks are the principal biological vectors of anaplasmosis, but the argasid tick *Ornithodoros savignyi*, can also transmit *A. marginale*. [20]. Reviews based on careful study of reported transmission experiments list up to 20 different ticks as capable of transmitting *A. marginale* [20]. These are: *Argas persicus, Ornithodoros lahorensis, Dermacentor albipictus, D. andersoni, D. hunteri, D. occidentalis, D. variabilis, Hyalomma excavatum, H. rufipes, Ixodes ricinus, I. scapularis, Rhipicephalus annulatus (formerly Boophilus annulatus), R. bursa, R. calcaratus, R. decoloratus, R. evertsi, R. microplus, R. sanguineus and R. simus*.

Rhipicephalus species are clearly important vectors of anaplasmosis in Africa and regions such as Australia, and Latin America, and some species of Dermacentor are efficient vectors in the USA [2].

Based on the fact that the geographic distribution of *R. (B.) decoloratus* in southern Africa is virtually the same as the area where bovine anaplasmosis is endemic, this tick is regarded as the main vector of *Anaplasma spp*. See Figure 3.

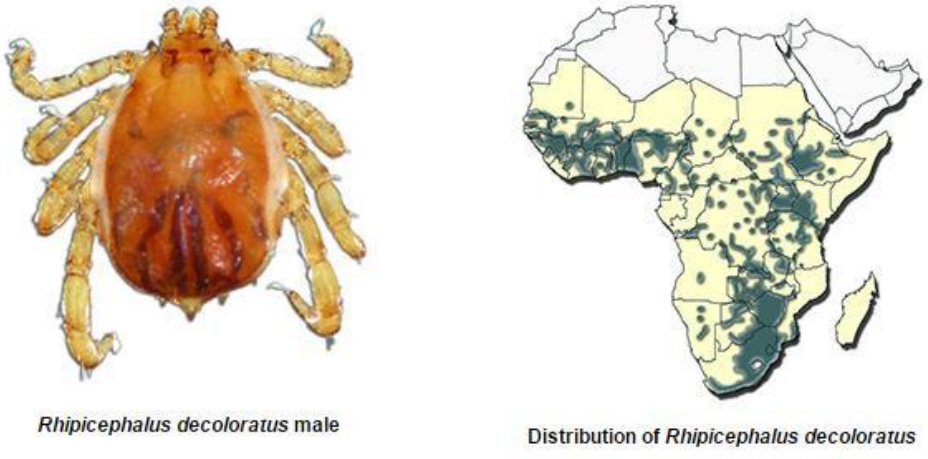


Figure 3: Rhipicephalus decoloratus and its distribution in Africa (source: http://www.afrivip.org/sites/default/files/Ticks-importance/rhipicephalus.html)

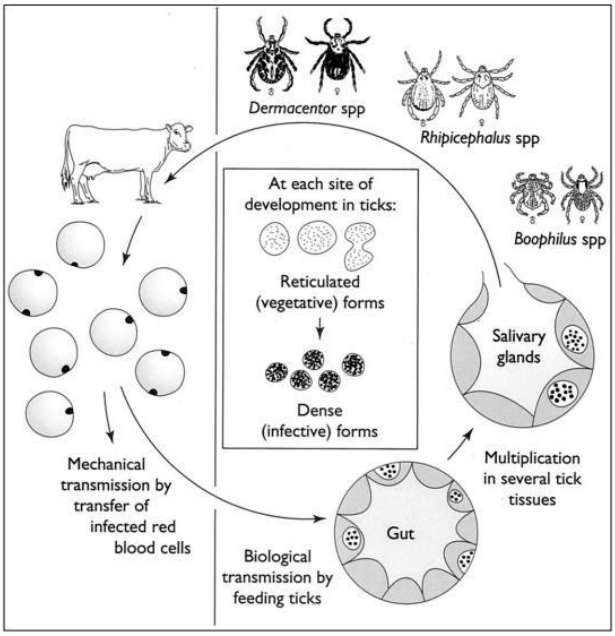


Figure 4: Schematic representation of the development cycle of *A. marginale* in cattle and ticks. (Source: 20)

In South Africa, the role that specific ticks play in the transmission of the disease has not been extensively studied. The one-host tick *B. decoloratus* has been incriminated circumstantially as being the most important vector, simply because occurrence of the disease is thought to correlate with the distribution of this tick. However, four other tick species (*B. microplus*, *R. simus*, *R. e. evertsi* and *H. m. rufipes*) have been shown experimentally to be capable of transmitting the infection, and their distribution largely overlaps that of *B. decoloratus*. These ticks are therefore also considered to be involved in the epidemiology of anaplasmosis in South Africa. [35]

The time required by feeding ticks to transmit the infection seems to vary between species. Adult *D. andersoni* have to feed at least six or seven days before transmission takes place, while adult *R. simus* may transmit the infection within 24 hours after infestation [37][35]

Intrastadial or transstadial transmission is the usual mode, even in the one-host Rhipicephalus species. Male ticks may be particularly important as vectors; they can become persistently infected and serve as a reservoir for infection. Experimental demonstration of vector competence does not necessarily imply a role in transmission in the field. However, Rhipicephalus species are clearly important vectors of anaplasmosis in countries such as Australia and countries in Africa, and Latin America, and some species of Dermacentor are efficient vectors in the United States of America (USA).

Mechanical transmission: At least 12 species of biting flies have been shown experimentally to have the potential of mechanically transmitting *A. marginale*, including stable flies (*Stomoxys calicitrans*), eight species of tabanids (Tabanidae – commonly called horse or deer flies) and three species of midges (Culicidae). [2]

Transplacental transmission has been measured at around 15 % by Potgieter and Van Rensburg [36], and as such is also likely to contribute to *A. marginale* epidemiology

Clinical Signs

The most marked clinical signs of anaplasmosis are anaemia and jaundice, the latter occurring late in the disease. This is the result of the fact that *A. marginale* primarily infects erythrocytes, and as part of the host response, these infected cells - along with considerable numbers of uninfected erythrocytes - are then destroyed by the reticuloendothelial system, this resulting into anemia and icterus [35]. Haemoglobinaemia and haemoglobinuria are not present, and this may assist in the differential diagnosis of anaplasmosis from babesiosis, which is often endemic in the same regions.

The severity of anaplasmosis is directly related to the age of the animal: in animals less than one year old it is usually subclinical; in yearlings and two-year-olds it is moderately severe; and in older cattle it is severe and often fatal. Differences in virulence between Anaplasma strains and the level and duration of the parasitaemia also play a role in the severity of the clinical manifestations. Generally, cases are presented for treatment when

the disease has advanced to the stage of the anaemic crisis. Anaemia is usually not clinically apparent until a loss of about 40 to 50 per cent of red blood cells has occurred. [35][42]

After a natural prepatent period, which generally varies between 15 and 36 days (with an average of 26 days), although it may be as long as 100 days, peracute, acute or chronic anaplasmosis may follow:

- **Peracute anaplasmosis**, rare and usually fatal, occurs most frequently in purebred animals and high-producing dairy cows. Animals succumb within a few hours of the onset of clinical signs. In addition to anaemia, milk production ceases, and there is excessive salivation, rapid respiration, irrational behaviour and signs of nervousness
- **The acute form** is essentially characterised by the pallor of mucous membranes, depression, inappetence, a decrease in milk production, general weakness, and a rapidly rising parasitaemia. In some animals, fever provides initial and persistent clinical evidence of anaplasmosis, but this is not consistent. The course of the acute disease is generally protracted and may last for two weeks or more before there is any evidence of improvement in the animal's condition
- **Chronic anaplasmosis** is manifested by slow recovery following acute disease, and it may persist for a period of between two weeks and three months. It is characterized by poor appetite, loss of weight and varying degrees of dehydration, anaemia and icterus which are usually milder than in the acute disease

A. marginale infections may induce temporary infertility in bulls, anoestrus in heifers, and abortions and neonatal anaplasmosis following foetal infection. The mortality rate, particularly in adult cattle of exotic breeds, may exceed 50 per cent [35]

Cattle that recover from acute disease usually remain persistently infected, often at microscopically undetectable levels (<107 rickettsia/ml). Throughout this persistent infection, they show cyclical rises in rickettsemia. These peaks in rickettsemia are believed to lead to the generation of antigenic variants, which escape immune control and multiply rapidly, before they stimulate a variant-specific immune response that brings the infection under control again. Due to this consistent stimulation, persistently infected cattle maintain a strong immune response against *A. marginale*, and are protected from disease when subsequently challenged with the homologous *A. marginale* strain. Unfortunately they also act as reservoirs of infection for naïve cattle [34].

Diagnosis

A diagnosis of bovine anaplasmosis may be made tentatively based on geographic location, history, season, signalment and presenting clinical signs and/or necropsy findings observed in infected animals.

However, microscopical identity of the agent in thin blood films stained with either Giemsa or proprietary stains is the traditional more accurate diagnostic method for clinical cases. In these smears, *A. marginale* organisms appear as dense, rounded, intraerythrocytic bodies approximately 0.3–1.0 µm in diameter situated on or near

the margin of the erythrocyte. *A centrale* is similar in appearance, but most of the organisms are situated toward the centre of the erythrocyte. It can be difficult to differentiate *A. marginale* from *A. centrale* in a stained smear, particularly with low levels of rickettsaemia.

Table 1 shows the tests recommended by the OIE for the different purposes: to demonstrate population or individual freedom, confirmation of clinical cases, etc.

Table 1: Tests recommended by the OIE [43]

Method	Population freedom from infection (non-vaccinated animals)	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases (2)	Prevale ce of infection-surveillance	Immune sattus in individual animals or populations post-vaccination
Microscope examination	-	+	-	+++	-	-
Agent identifictaion						
PCR	-	+++	-	+++	-	-
DETECTION OF IMMUNE RESPONSE						
CAT	-	-	-	-	+ -	+
ELISA	+++	+	+++	-	+++	+++
IFAT	+	-	-	-	++	++
CFT	-	-	-	-	+	-

Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose. Although not all of the tests listed as category +++ or ++ have undergone formal validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable. Agent id. = agent identification; CAT = card agglutination test; CFT = complement fixation test; ELISA = enzyme-linked immunosorbent assay; IFAT = indirect fluorescent antibody test; PCR = polymerase chain reaction

The gold standard for the demonstration of *A. marginale*-free blood is the sub-inoculation of blood from the suspect animal into a splenectomized calf that is highly susceptible to infection [35][43]. If the donor is infected, *A. marginale* will be observed in smears from the splenectomized calf generally within 4 weeks, but this period may extend up to 8 weeks. However, this method is costly and raises welfare issues, as the splenectomized calves become very ill after sub-inoculation of infected blood and often have to be euthanized [43]. For these reasons, it would not be feasible to use sub-inoculation of splenectomized calves as the gold standard for the validation of assays. Thus, older tests have typically been validated using microscopic detection of *A. marginale* or comparison with other serology results, and newer ELISA tests have usually been validated using PCR methods that have not been formally validated themselves.

Lateral flow assays have been, as in many tropical diseases a desired option, especially if they could help in differentiating the disease with others having similar signs, at field level.

Canadian researchers from Canadian Food Inspection Agency have developed a lateral flow assay (LFA) for the rapid detection of bovine antibody to *A. marginale* [28]. The assay uses a recombinant peptide of MSP5 as the antigen and a monoclonal antibody specific for bovine IgG1 conjugated with colloidal gold beads for detection. Of the 114 samples that were selected based on positive identification of the organism in blood smears, all were positive by LFA, cELISA and semi-nested PCR (Se = 100% for all three tests). Samples from Canadian sources (n = 524) were all negative on the cELISA using 30% inhibition as a cut-off and PCR (n = 40, randomly selected), but 11 sera gave false-positive reactions on the LFA (Sp = 97.9%). Of 113 samples from non- Canadian herds in bovine anaplasmosis-endemic areas, 53 were positive on the cELISA and 50 were LFA positive. Using the cELISA as a reference, the sensitivity of the LFA would be estimated at 94% [28].

Incidence and Prevalence in Selected Countries

Global

As stated earlier bovine anaplasmosis has a wide distribution and occurs in tropical and subtropical regions worldwide (approximately 40° N to 32° S), including South and Central America, the USA, southern Europe, Africa, Asia and Australia.

Reporting of the disease however, as is the case for many endemic diseases in Africa and Asia, is very irregular and not necessarily representing the full picture. Since there is no evidence that the disease can be eradicated from infected countries, all countries having reported the disease at a certain stage can be considered harbouring the infection.

Regional

1- Source: OIE.

Data of outbreaks reported to the World Animal Health Organization [43] are shown in Tables 2 and 3. Data are not always reliable, as many countries do not seem to report, or to be reporting consistently over time.

http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail. Similar information but presented in a different manner can be seen in Annex 1.

- No information, + Present but quantitative data not known, ? Disease suspected

Table 2: ASIA- Number of Bovine Anaplasmosis outbreaks reported to the OIE between 2005-2015 (Numbers given only for the target countries) Source: OIE.

Country	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Asia											
Bangladesh			+	+	+	+	+	+	+	+	
India	14	14	13	14	16	29	27	27	38	108	
Indonesia	+	+	+	+	+	+	+	+	+		
Myanmar (Burma)	0	0	0	0	4	3	5	0	3	23	
Nepal	2	2	0	0	0	4	3	4	0	0	
Vietnam											

Table 3: AFRICA- Number of Bovine Anaplasmosis outbreaks reported to the OIE between 2005-2015 (Numbers given only for the target countries). Source: OIE.

Country	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
West Africa											
Mali	0	0	0	0	+	0	0	0	0	0	
Senegal	1	0	0	0	0	0	0	0	0	0	
East Africa											
Ethiopia	+	0	+	+	+	+	+	+	0	0	
Kenya	0	+	1	+	+	+	+	2	+	5	
Rwanda	0	+	+	+	+	+	+	+	+	+	
Tanzania	30	35	332	114	69	61	45	19	32	45	

Uganda	+	3	+	+	1	+	+	+	+	+	
Southern Africa											
Madagascar	10	9	13	0	0	8	8	8	10	7	
Malawi	1	1	1	4	5	3	0	6	0	0	
Mozambique	4	14	7	7	4	15	7	11	8	10	
South Africa	161	96	60	102	91	77	59	43	25	14	
Zambia	0	80	90	182	291	237	0	162	181	170	

2- Source: AU-IBAR.

For the African continent, the number of outbreaks reported to AU-IBAR is included in the Pan African Animal Resources Year Book. (**http://www.au-ibar.org/pan-african-animal-resources-yearbook?showall=&limitstart**=) and can be seen for the countries of interest in Table 4 below.

Table 4: Number of Bovine Anaplasmosis outbreaks reported to the AU-IBAR from 2005 to 2015 (numbers given only for the target countries). Source: AU-IBAR Year Books.

Country	2005*	2006**	2007	2008	2009	2010	2011	2012	2013	2014	2015
West Africa											
Senegal				1							
East Africa											
Kenya			31	57	+	+	88	76	11	9	
Rwanda											
Tanzania			441	+	+	+	38	20	31	47	
Uganda				10	10	35			5	2	

Southern Africa											
Madagascar			1	+	+	+	18				
Malawi			1		+			7			
Mozambique			5	15	3	12	7	2	11	5	
South Africa			60	102	35	81		55	26	15	
Zambia			90	+	48	2		43	174	122	

*AU-IBAR didn't start yet producing data for bovine anaplasmosis
**No individual country report available; 7 countries reported to a total of 334 outbreaks

Prevalence data by country:

The information below has been obtained from PubMed and internet engine search. The information by country is extremely limited, as it tends to focus on the outbreaks, and very little countrywide surveys are conducted.

ASIA

Bangladesh

- Although Bovine Anaplasmosis and *A. marginale* have been reported to be prevalent in Bangladesh since 1976, with prevalence reaching 70% in certain districts [4], reporting to the OIE has been limited
- In the study conducted by Belal et al. [4] in the Sirajganj District, which is the most important dairy belt area of Bangladesh with a high density of cattle population, they found an overall prevalence of 25.82%, with prevalence in older animals reaching 34.19%.

India

- Bovine anaplasmosis is recognised as one of the most economically important disease of livestock in India, ranking in the top 10 most important livestock diseases in the country in 2010 [11].
- Clinical anaplasmosis was first recorded in Indian cattle from the State of Odisha. Subsequently, *A. marginale* infection was detected in livestock of Uttar Pradesh, Punjab, Haryana, Tamil Nadu, Karnataka, Jammu and from parts of north and central India. In recent times, anaplasmosis has been recorded in Jammu, Karnataka, Haryana and Tamil Nadu.

- Reporting to the OIE has covered the following areas:
    - Goa
    - Jammu & Kashmir
    - Kerala
    - Orissa
    - Pondicherry
    - Rajasthan
    - Uttar Pradesh
    - West Bengal
    - Punjab
- In a study published by Ashuma et al. (2013) a 37.5% more prevalence of *A. marginale* was recorded by PCR method in dairy animals of Punjab
- It can be concluded that bovine anaplasmosis is of high economic importance in India.

Indonesia

- As per their 2005 OIE report, typical clinical cases of anaplasmosis are found in cattle and some buffaloes in the provinces of West Sumatra, Riau, Jambi, West Java, Central Java, Yogyakarta, East Java, South Kalimantan and Southeast Sulawesi. 768 cases were reported in 2005
- Indonesia has been reporting bovine anaplasmosis during the past 10 years.
- In a study on feeder cattle imported to Indonesia from Australia in order to increase local production and meet increasing demand, a prevalence of 60.3% was found in sampled cattle [41], although these animals did not show clinical disease

Myanmar

On OIE reports, the administrative regions of Yangon and Shan State are mentioned as having the disease.

Nepal

Although incidence is reported to the OIE, no prevalence data could be found

Vietnam

- Based on OIE reports, the disease has been suspected over the 10 year period covered, but never reported
- In a study on dairy cattle around Hanoi, northern Vietnam, blood samples were examined with commercially available Svanovir®Elisa's for the presence of *A. marginale* specific antibodies, and a prevalence of 28% was found (**Geurden et al. 2008**)

AFRICA

Burkina Faso

No data could be found

Cote d'Ivoire

No data could be found

Ethiopia

- A study on 384 blood samples collected from ruminants present in Debre-Zeit town and surrounding peasant associated areas found a prevalence of 6.3% to Anaplasmas, of which 2.1% was *A. marginale* [38]
- In a survey reported by ILRI, not dated (**https://www.ilri.org/InfoServ/Webpub/fulldocs/Dolanm/ethiopia.htm**), 2434 blood smears and 242 serum samples were collected from 21 state and private dairy farms to determine the prevalence of tick-borne infections, mainly in dairy cattle. Over 90% of sera tested were positive for *A. marginale.*

Kenya

- A recent study conducted in intensively studied cohort of indigenous calves in western Kenya where calves were recruited close to birth and monitored for the presence of infectious disease for up to 51 weeks, using the reverse line blot hybridization assay on the remaining 453 animals, showed a prevalence of 42.7%, part of co-infection with other haemoparasites [29]
- In a paper published in 2014 by the same group where the same calves were monitored for seroconversion, it was shown that 50% of the final 453 animals seroconverted to *A. marginale*, when tested with the species-specific antibody-capture ELISAs [16]

- The high seroconversion rate and high prevalence noted in this important study denote the significant importance of *A. marginale* in Kenya, though the study focused on Western Kenya.
- In an earlier study in wildlife and domestic species in wildlife-livestock interface areas of Kenya by [27], where a competition inhibition ELISA assays was used on samples from different game animals, the prevalence detected is summarised in Table 5 below.

Table 5: Seroprevalence of antibodies to *Anaplasma spp*. In various species at the wildlife/livestock interface in Kenya [27]

Species	District	No. positive
Eland	Machakos Nakuru Mombasa	12/12 (100 %) 3/4 (75 %) 2/2 (100 %)
Blue wildebeest	Machakos	56/58 (96.5 %)
Kongoni	Machakos	112/120 (93.3 %)
Impala	Machakos	7/7 (100 %)
Thomson's gazelle	Machakos	6/8 (75 %)
Grant's gazelle	Machakos	4/5 (80 %)
Giraffe	Machakos Nakuru	3/3 (100 %) 11/13 (84.6 %)
Plains zebra	Machakos	8/11 (72.7 %)
Cattle	Thika Machakos Laikipia	29/29 (100 %) 31/31 (100 %) 82/88 (93.2 %)
Sheep	Thika Machakos	24/30 (80 %) 20/20 (100 %)
Goats	Machakos	17/20 (85 %)

- In another study, a cross-sectional survey conducted by Gachohi and associates on haemoparasitic diseases of small holders in Kenya have mentioned the prevalence of Anaplasma marginale to be 52-64% (**Gachohi et al., 2010**)

Madagascar

Although the disease is known to occur and reports are made to the OIE, no prevalence studies could be found.

Malawi

Although the disease is known to occur and reports are made to the OIE, no prevalence studies could be found.

Mali

No information available.

Mozambique

- In a serological survey based on card agglutination test for *A. marginale*, conducted on 478 communal calves (4 to 15 months old) of the north western province of Tete, Mozambique, a prevalence of 63% was determined, ranging from 34.4% in one of the 6 districts to 87.3% in another [1]
- Mozambique has been reporting bovine anaplasmosis regularly to the OIE and the AU-IBAR.

Rwanda

Although no specific studies could be found, bovine anaplasmosis is highly prevalent in Rwanda, based on reports to the OIE and on high occurrence of vector ticks the prevalence [31].

Senegal

Although recognised to be present, no recent literature on bovine anaplasmosis in Senegal could be found.

South Africa

- There are several reports on the prevalence and occurrence of bovine anaplasmosis in South Africa.
- A 2007 study reported by Mtshali [25] conducted in two regions of the Free State Province, a prevalence 44% to 98%, similar in both regions, was found.
- In another study reported by Mutshembele et al. [26], using aMSP1a PCR on 250 blood samples collected countrywide, prevalence ranging from 60 to 100% were recorded.

Tanzania

- A cross-sectional serological survey of *A. marginale* conducted on 200 randomly selected smallholder farms in each of the Tanga and Iringa Regions of Tanzania, Swai et al. [40] assessed antibodies against *A. marginale* in sera from dairy cattle of all ages, sexes and breeds by ELISA. Antibodies to *A. marginale* were present in cattle throughout the study areas and the overall prevalence was 20% for Tanga and 37% for Iringa.
- These results are in line with the regular reporting by Tanzania to the OIE and the AU-IBAR over the past 10 years.

Uganda

- A recent review by Mikael Palmfjord [23] indicates that in serological analyses of Anaplasma spp. in Uganda, sero-prevalences have ranged between 30-60 % (Table 6). He also mentions that another recently published study in Central and Western Uganda showed an overall microscopic prevalence of Anaplasma spp. of 14.4 %.

Zambia

- Jongejan et al. [15] found prevalence by CAT on samples collected throughout Zambia of 14.7% to 38.6%. By retesting 200 of the sera by ELISA the sero-prevalence was 1.5 to 2.3 times greater with ELISA than with the CAT.
- In a 2009 PhD thesis by Simmunza [39] which included an Epidemiological analysis of tick-transmitted diseases of cattle in Central, Lusaka and Eastern Zambia, a total of 637 cattle were sampled and of these, 422 were from Eastern Province, 151 from Central province. The table below provides a summary of prevalence determined using the 16S rRNA gene semi-nested PCR assays. Prevalence of Anaplasma spp., essentially *A. marginale* was one of the highest of all the TBD agents tested, varying between 40 and 83%.

Table 6: Recorded sero-prevalence and microscopic prevalences of Anaplasma spp. in Uganda Source: 23

Study	Study area	Method	Recorded prevalence	*Anaplasma* spp
Magona & Mayende, 2001	Tororo and Soroti, Central eastern Uganda.	Giemsa-stained blood smears	13.3%	*A. marginale*
Rubaire-Akiiki *et al.*, 2004	Mbale district Central eastern Uganda	I-ELISA (Morzaria *et al.* 1999)	– 30 % in fenched in lowland < 60 % in free-range in lowland	*Anaplasma* spp.
Kabi *et al.*, 2008	Soroti district Central eastern, Uganda	I-ELISA (Svanova Biotech AB Uppsala)	58 % Ankole cattle 57 % Zebu/Nakidi cattle	*Anaplasma* spp.
Angwech, 2011	Gulu district, Nothern Uganda	Giemsa-stained blood smears	10.4 %	*Anaplasma and A.centrale*
Kabuusu *et al.*, 2013	Queen Elisabeth National park, Western Uganda	Giemsa-stained blood smears	15%	*A. marginale*
Matovu *et al.*, 2014	Central and Western Uganda	Giemsa-stained blood smears	14.4 % overall 24.8 % western 6.0 % central	*A. marginale* and *A. centrale*

Table 7: Prevalence (%) of tick-borne pathogens of cattle for the dry and wet season in Central and Eastern Zambia Source: 39.

	Eastern province			Central province			Lusaka province			Overall		
	Dry season	Wet season	*p value*	Dry season	Wet season	*p value*	Dry season	Wet season	*p value*	Dry Season	Wet season	*p value*
n	422	211		151	56		64	82		637	349	
Ba. bovis	10.90	32.70	< 0.001*	4.67	1.78	0.686	32.81	9.76	< 0.001*	11.62	22.35	< 0.001*
Ba. bigemina	0.24	11.85	< 0.001*	1.32	0.00	1.00	0.00	14.63	< 0.001*	0.47	10.60	< 0.001*
T. parva	22.04	30.33	0.023 *	25.83	14.29	0.078	14.06	40.24	< 0.001*	22.14	30.09	0.006 *
T. taurotragi	21.33	36.49	<0.001 *	23.18	17.86	0.410	28.13	37.80	0.219	22.45	33.81	< 0.001 *
T. mutans	48.82	83.89	< 0.001*	66.89	39.29	<0.001*	70.31	74.39	0.584	55.26	74.50	< 0.001*
Anaplasma spp	40.76	63.51	< 0.001*	76.83	48.21	<0.001*	62.50	52.44	0.223	51.49	58.45	0.036 *
E. ruminantium	5.45	18.96	< 0.001*	37.75	33.93	0.613	45.31	29.27	0.045*	17.11	23.78	0.011 *

Economic and Social Impacts at Global and Regional Levels, and in Selected Countries

Throughout the tropical and subtropical areas of the world where anaplasmosis occurs, the disease is a major constraint to the cattle production in many countries.

For example, in a 2003 publication, Kocan et al. cited annual losses in beef cattle in the United States as a result of anaplasmosis morbidity and mortality being estimated to be over $300 million per year. In the same paper, she mentions that in Latin America those losses were calculated to be approximately $800 million per year.

Most economic impact studies covering bovine Anaplasmosis are conducted for all Tick-borne parasitic diseases. The study and report by Minjaw B et al., 2003 provides more information on the impact of ticks and tick-borne diseases on the livelihoods of small-scale and marginal livestock owners in India and eastern and southern Africa.

A more recent report by Kivaria, 2006 in Tanzania, estimated the direct annual losses due to bovine anaplasmosis to amount to 47.3 million USD [17].

In India, bovine anaplasmosis is considered as one of the top 10 diseases of cattle. [11]

Based on the work by Minjaw et al. [24], the number of animals at risk of bovine anaplasmosis based on the presence of the vectors has been estimated to be very high, over 114 million, in Sub-Saharan Africa, as illustrated in Figure 5. This risk map was developed through a DFID project, in 2003, conducted jointly with ILRI.

To create a disease risk map, the predicted distribution maps of all of the ticks responsible for the transmission of bovine anaplasmosis were combined. The resulting map, in Figure 5 excludes any predictions of habitat suitability (at all levels of probability) that were more than 3 degrees from an observed value. In this way, the actual risk of disease was determined by the observed presence of the vectors, and areas in which the habitat might be suitable but the vectors absent were excluded.

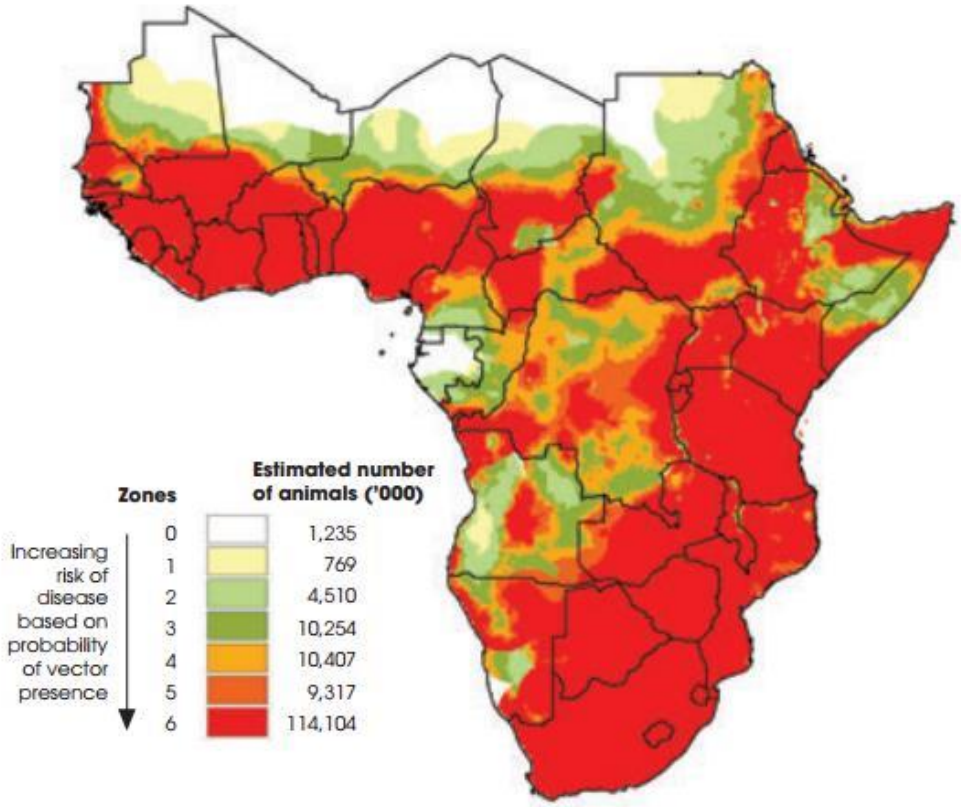


Figure 5: Distribution of bovine anaplasmosis risk based on probability of vector presence [24]

Disease Prevention and Control Methods

Treatment (Control)

Control of Bovine Anaplasmosis varies according to regions and, depending on whether outbreaks are occurring in a known enzootic area, or as focal isolated events in non-enzootic regions, control measures that could be implemented will consist of the following approaches or their combination:

- Maintaining an *A. marginale*-free herd
- Vaccination
- Tick control
- Treatment with tetracycline during acute disease
- Administration of low-level tetracycline for prevention of clinical disease

Tick control

Arthropod control is not practical in many areas and may only partially prevent against *A. marginale* transmission, which occurs both by mechanical transmission of infected blood via insects and fomites and by biological transmission via ticks.

In regions where anaplasmosis is endemic, such as in Sub-Saharan Africa, its eradication followed by the implementation and long-term maintenance of strict tick control programmes (to control it and other endemic tick-borne diseases, such as heartwater, babesiosis and anaplasmosis) is not generally recommended. Stringent tick control would render the cattle population susceptible to several other tick-borne diseases with potentially catastrophic consequences.

In addition to problem associated to tick resistance to acaricides, a further complicating factor in Southern and Eastern Africa is that populations of wild ruminants sustain tick populations and that some are probably reservoir hosts to *A. marginale*.

For these reasons it is generally recommended in Southern and Eastern Africa, that a stable disease situation be pursued by allowing natural exposure of calves to tick-borne diseases, including anaplasmosis, during the period when they are naturally resistant or protected by passively acquired maternal antibody.

Chemotherapy

The treatment of clinical anaplasmosis in cattle requires, firstly, the suppression or elimination of the parasite (specific treatment) and, secondly, the alleviation or prevention of secondary complications and the hastening of recovery (symptomatic or supportive treatment). Different products are used as summarised in Table 9 below.

Tetracycline antibiotics are by far the predominant treatment for bovine anaplasmosis. Repeated doses of tetracycline can eliminate persistent *A. marginale* infections, although complete elimination is not always achieved.

Imidocarb and Enrofloxacin have also shown good results, but they are not commonly used [35].

Tetracycline is most effective in the earlier stages of the disease: it can be difficult to catch the disease early enough in range cattle [20]. Therefore, it is sometimes used prophylactically, particularly in the US [20]. Such frequent use of antibiotics has the potential to cause selection of resistant strains, but to date this has not been reported as a problem.

Tetracycline is also used in some regions in the “Infection-treatment” method. This procedure involves inoculation of cattle with *A. marginale*-infected erythrocytes followed by treatment with low doses of tetracycline drugs during the initial appearance of patent infection. The cattle then become persistently infected without experiencing acute anaplasmosis and are subsequently immune to challenge exposure with the same or different isolates. This approach requires careful monitoring to ensure acute disease does not develop, and so can be unsuitable for large herds of cattle.

While antibiotic treatment can clear and sterilise the infection, there are a number of concerns worth noting;

- Cattle in which infections have been eliminated shortly after primary infection exhibit no residual immunity upon challenge with *A. marginale*. In contrast, cattle cleared of carrier infections are susceptible to reinfection but show resistance to clinical anaplasmosis for periods ranging from a few to 30 months following parasite elimination [35]
- The withholding period in antibiotic-treated cattle before they can be used for meat or milk can be a problem for farmers, particularly with long-lasting oxytetracycline preparations
- While antibiotic resistance for Anaplasmosis has not been described, the frequent use of antibiotics in Africa, where substandard and counterfeit drugs are commonly found in many countries, could impact negatively on other concurrent or secondary infections

Immunisation

Since the first description of *A. marginale* and *A. centrale* by Arnold Theiler in 1910 and the subsequent development of an *A. central* based blood vaccine, there has been no new effective alternative widely used to immunise cattle, besides inactivated vaccine used in limited fashion in the USA. Vaccination has been an economical and effective way to control bovine anaplasmosis wherever it has been applied. Vaccines for the control of anaplasmosis can be divided into two major types: live and killed vaccines. Both types of vaccines rely on the use of *A. marginale* or *A. centrale* from infected bovine erythrocytes as the antigen source.

They both induce protective immunity that mutes or prevents clinical disease, but neither type prevents cattle from becoming persistently infected with *A. marginale*.

Killed vaccine

Killed bovine anaplasmosis vaccines are almost exclusively made and used in the USA. Developed in the 1960s, and marketed commercially until 1999 when most of them got withdrawn, they have advantages and disadvantages summarised in Table 8 below.

Table 8: Advantages and disadvantages of the killed bovine anaplasmosis vaccines

ADVANTAGES	DISADVANTAGES
• They have low risk of contamination with undesirable infectious agents, • Can be inexpensively stored and • Generally cause minimal post-inoculation reactions.	• The need for yearly boosters, • High cost of purification of *A. marginale* from erythrocytes and • Lack of cross protection among isolates from widely separated geographic areas. • The protective immunity afforded is usually less than that of live vaccines.

Two commercial bovine anaplasmosis killed vaccines were manufactured and commercialised in the US by Fort Dodge (Fort Dodge Laboratories, Fort Dodge, Iowa) and Mallinkrodt (later Schering-Plough) under the commercial name “Anaplaz®”, and “Plazvax®” respectively. They both got discontinued in 1999.

Currently the only manufacturer, who has received USDA approval to commercialise a killed *A. marginale* vaccine is the US is the University Products LLC of Baton Rouge. (**http://www.anaplasmosis.com/aboutus.html**).

Live vaccines

Live vaccines have been used against bovine anaplasmosis for over a century, from the time Sir Arnold Theiler first identified *A. marginale* and *A. centrale*. They are still today the most widely used type [19]. As live vaccines, most of which are based on *A. centrale*, lead to a persistent infection in immunized cattle, they provide with lifelong protection against clinical and severe disease. They produce a stronger immune response than killed vaccines, with the generation of antigenic variants during persistent infection leading to a broader immune response [34]. This type of vaccine is further discussed in the following section.

Research toward a better understanding of the *A. marginale* organism, its infection, and host pathogen interaction have led to the development of several vaccine strategies and experimental candidates, none of which have gone beyond proof of concept stage.

Table 9: Bovine anaplasmosis vaccines developed to date

Vaccine	Dosage, Administration and Withdrawal Times	Countries	Life Stages	Adverse Affects
Anaplasma centrale	Prepared from blood of infected splenectomized calves. Chilled or Frozen forms. Administered as a single vaccination.	South Africa, Australia, Israel, Argentina, Uruguay,	All Stages	Can cause severe clinical reactions. Risk of contamination with pathogens unless high standards applied.
inactivated Anaplasma marginale vaccine	2 doses 4 weeks apart with annual booster. Withdrawn in 1998.	USA	All Stages	May cause neonatal isoerthrolysis in calves from some vaccinated Dams.
killed Anaplasma marginale vaccine	2 doses 4 weeks apart with annual booster. Withdrawn in 1998.	USA	All Stages	
Louisiana State University experimental vaccine	2 doses given 4 weeks apart, with yearly booster. Given subcutaneously. Vaccine does not prevent infection but aids in prevention or reduction in the severity.	USA	All Stages	
modified live ovine Anaplasma marginale vaccine	2 ml intramuscularly in a single dose. No longer available outside California, USA.	South America; Australia	All Stages	May have severe clinical reactions especially in cattle over 2 years of age. Risk of contamination with pathogens unless high standards applied.

Disease situation and government policies by country

Tables 10 and 11 below have been completed with the information received so far from the questionnaires sent to the DG and DVS of the countries of interest. This information will be updated and completed once the results from the different countries is received. The list of the respondents can be seen in Annex 2.

Table 10 covers the disease situation (if it is notifiable or not), the presence of official surveillance and/or control programs, and the treatment situation. Table 11 refers to the vaccination situation.

The definitions that were given to the respondents are:

[1]Surveillance: is the systematic ongoing collection, collation and analysis of data and the timely dissemination of information to those who need to know so that action can be taken.

[2]Control: a programme which is approved, and managed or supervised by the Veterinary Authority of a country for the purpose of controlling a vector, pathogen or disease by specific measures applied throughout that country, or within a zone or compartment of that country.

Table 10: Official status, official programs for Bovine anaplasmosis in the countries of interest Information provided by the questionnaire sent to the DG/DVS as part of this monograph.

Country	Notifiable (yes/no)	Official surveillance[1] program (yes/no) (if yes, active or passive)	Official control[2] program (yes/no)	Treatment (Chemotherapy)	
				Treatment authorised (yes/no)	Frequently practiced (yes/no)
ASIA					
Bangladesh					
India					
Indonesia					
Myanmar (Burma)	No	No	No	No	Yes
Nepal					
Vietnam					
AFRICA					
Burkina Faso					
Côte d'Ivoire (Ivory Coast)	Yes	Passive, but active if outbreaks	No	Yes	When animals are sick
Ethiopia					
Kenya	Yes	Yes	No	Yes	Yes

Madagascar					
Malawi	No	No	Yes	Yes	Yes
Mali	-	-	-	-	-
Mozambique					
Rwanda					
Senegal					
South Africa					
Tanzania	No	Yes, passive	Yes	Yes	Yes
Uganda					
Zambia					`

Table 11: Official status, official programs for Bovine anaplasmosis in the countries of interest Information provided by the questionnaire sent to the DG/DVS as part of this monograph.

Country	Vaccination			
	Compulsory vaccination (yes/no)	Who pays for the vaccine (Government, farmers, combination, others-specify)	Who delivers the vaccine (official, private vaccinators or both)	Species vaccinated (cattle, sheep, goats, pigs, poultry)
ASIA				
Bangladesh				
India				
Indonesia				
Myanmar (Burma)	No	-	-	-

Nepal				
Vietnam				
AFRICA				
Burkina Faso				
Côte d'Ivoire (Ivory Coast)	No	-	-	-
Ethiopia				
Kenya	N/A	N/A	N/A	N/A
Madagascar				
Malawi	No	N/A	N/A	N/A
Mali	N/A	N/A	N/A	N/A
Mozambique				
Rwanda				
Senegal				
South Africa				
Tanzania	No	Not done	Not done	N/A
Uganda				
Zambia				

- : No information provided in the questionnaire.

Vaccines Available

Although several approaches have been tried to immunise cattle against anaplasmosis, the oldest and to date probably still the most effective and widely used method of prophylaxis against anaplasmosis is vaccination with Theiler's *A. centrale* isolate, as blood vaccine [43]. However, *A. centrale*, although generally a mild pathogen, can cause severe clinical reactions following vaccination.

At Onderstepoort veterinary Institute (OVI) where the South African vaccine antigen is produced, cattle born and raised under strict tick-free (quarantine) conditions and tested free from blood-transmissible pathogens are splenectomized prior to infection with the Onderstepoort *A. centrale* vaccine parasite. The intensity of the infections in the animals is measured and blood is collected in anticoagulant at the acute stage of infection with these parasites. The blood is then diluted to contain a standardized number of parasites per dose.

OVI supplies bulk blood vaccine to Onderstepoort Biological Products (OBP) where the vaccine is bottled into five-dose containers, snap-frozen before storing in bulk liquid nitrogen containers. Frozen vaccines are produced in monovalent form. The vaccine is packed, and subjected to strict quality control testing before it is released for issue on demand.

The challenges associated with the logistics of distributing the frozen blood vaccine from OVI/OBP have limited its distribution in certain parts of the country and prevented its commercialisation beyond South Africa. Sporadic limited sales are done outside South Africa, especially in other Southern African countries such as Zambia, Zimbabwe, Namibia and Mozambique on a limited scale (personal communication OBP Export sales manager).

While the South African vaccine is supplied in a frozen form, Australia produces a Trivalent chilled vaccine (**https://www.daf.qld.gov.au/__data/assets/pdf_file/0008/61388/Tick-Fever-A2-Trivalent-Tick-Fever-Vaccine-Specifications.pdf**), which is distributed within the 4 days of its shelf life.

Other countries that produce the frozen vaccines made of the *A. centrale* originating from the Theiler vaccine are Argentina, Uruguay and Israel. Malawi and Zimbabwe had produced the vaccine for a while and have discontinued since.

Vaccination against bovine Anaplasmosis is not practiced in Asia, and no vaccines are available in affected countries.

Table 12 below summarises the strength, weaknesses, threats and opportunities with the current bovine anaplasmosis blood vaccine used, both as a monovalent or a bivalent vaccine.

Table 12: Strengths, weakness, threats and opportunities of the commercially available Anaplasmosis vaccines.

Vaccine type	Strengths	Weakness & Threats	Opportunities
A. centrale based live blood vaccine	• Cross protection to most *A.marginale* • Proven ability to be used in the control of bovine anaplasmosis. • Leads to high production of IgG, high cross-reactivity within that IgG response, a strong IgG2 bias, and high opsonophagocytic activity (Kenneil; 2015)	• Limited efficacy • Very difficult to produce, relies on old technology • Difficult to validate production process in modern vaccinology (produced in cattle). • Production in live cattle carries the risk of transmission of other blood borne pathogens.	• Cell culture to replace cattle; • Better understanding of mechanisms that make A. centrale better vaccine than attenuated A.marginale may lead to better alternatives. • Concerns on antibiotic resistance will lead to more reliance on vaccine, hence creating new opportunities for the vaccine.

Commercial vaccines manufactured in Africa and Asia

The only vaccine produced in Africa and the target regions of Asia is the OBP, and summarized in Table 13 below.

Table 13: Characteristics of the BA vaccine produced by OBP (source: www.vetvac.org)

Manufacturer	Onderstepoort Biological Products Ltd.		
Countries of distribution	Namibia, South Africa		
For vaccination of the following animals	Cattle		
Vaccine claims	Pathogen name	Type/Strain	Component description
	Anaplasma marginale		Live
Other information			
Dosage (ml)	1	**Route(s) of administration**	Intramuscular
Use with pregnant animals	Pregnant cows may abort and should therefore be vaccinated only after calving.	**Duration of immunity (months)**	
Reconstitution		**Known side effects**	The anaplasmosis vaccine reactions usually set in from the 4th to the 6th week after inoculation and last for approximately 2 weeks. The peak of the reaction may be accompanied by fever (40 °C or higher) and symptoms such as poor appetite, decreased milk production, constipation, anaemia and jaundice.
Adjuvant(s)		**Withdrawal period (days)**	7
Genetically modified?	no	**Packaging**	Bottles of 5 doses

Storage	-70 ºC. May only be kept on dry ice or in liquid nitrogen (temperatures below -70°C). Vaccine which on receipt is completely thawed, is ineffective an	**Shelf life (months)**	
Prescription		**Approval reference(s)**	Reg No. G1106 (Act 36/1947, South Africa), NSR 0569 (Namibia)
Notes & comments	In those areas of the country where anaplasmosis occurs, all calves should be vaccinated at approximately 6 months of age (between 3 and 9 months). Cattle should have developed immunity on average 2 months after vaccination.In anaplasmosis areas where vaccinated cattle are exposed to infected ticks, they will develop life-long immunity.		

Commercial vaccines imported into Africa

The information summarised in Table 14, is based on a questionnaire send to the Director of Veterinary Services office and regulators of the countries of interest. Note that some vaccines might have been imported under DVS dispensation, and they are not necessary licensed in the country.

Given the fact that no Asian countries vaccinate against Bovine anaplasmosis, neither do most African countries, there is very little imports of this vaccine

Table 14: Bovine anaplasmosis vaccine imported into the different countries

Country	Vaccine name	Strain or type	Country of origin	Doses imported 2015	Doses imported 2014	Doses imported 2013	Doses imported 2012
ASIA							
Bangladesh							
India							
Indonesia							
Myanmar (Burma)	-	-	-	-	-	-	-
Nepal							

Vietnam							
AFRICA							
Burkina Faso							
Côte d'Ivoire (Ivory Coast)	-	-	-	-	-	-	-
Ethiopia							
Kenya	-	-	-	-	-	-	-
Madagascar							
Malawi	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Mali	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Mozambique							
Rwanda							
Senegal							
South Africa							
Tanzania	-	-	-	-	-	-	-
Uganda	-	-	-	-	-	-	-
Zambia							

- : No information provided in the questionnaire.

Characteristics of Ideal Vaccine Candidates for Smallholders

Table 15: Target Product Profile (TPP) Bovine Anaplasmosis vaccine – Proposal:

	Attribute	Minimum (current available vaccine)	Ideal
1	Antigen	Immunogen with protective antigens of *A. marginale* or *A. centrale* that protects against *A.marginale* infection	Immunogen capable of providing full protection in cattle against *A. marginale* infection
2	Indication for use	For active immunization of cattle & water buffaloes	For active immunization of cattle, water buffalos and all susceptible animals
3	Recommended species	Cattle, Water buffaloes	All *A. marginale* susceptible livestock
4	Recommended dose	2 ml	1 ml
5	Pharmaceutical form	Reconstituted injectable solution/suspension (freeze-dried vaccine) or ready to use solution (inactivated vaccine)	Ready to use solution/suspension
6	Route of administration	intramuscular	SC, Intramuscular or pour on
7	Regimen - primary vaccination	Single dose	Single lifetime dose

8	Regimen - booster	Single annual booster	Lifelong immunity after primary vaccination
9	Epidemiological relevance	Protection against all geographically distinct strains of *A.marginale*	Protection against RVF and prevention of virus transmission
10	Recommended age at first vaccination	Animals over 3 months: one injection	From 1-2 months of age
11	Onset of immunity	2-3 weeks following primary vaccination	One week following primary vaccination
12	Duration of immunity	At least 1 year	Lifelong immunity
13	Expected efficacy	To prevent disease & prevent mortality.	To prevent infection and transmission. No disease & no mortality in vaccinated animals after virulent challenge.
14	Expected safety	In animals under 6 months of age, a transient pyrexia reaction can occur. A transient nodular reaction of varying importance may appear at the injection site, it progressively disappears within 1 to 2 months. Only vaccinate pregnant animals on emergency.	No post-vaccinal reactions at any age. Safe for pregnant animals. No carrier form in vaccinated animals
15	Withdrawal period	Nil	Nil
16	Special requirements for animals	Do not vaccinate un-healthy animals	Do not vaccinate un-healthy animals DIVA
17	Special requirements for persons	None	None
18	Package size	50 doses	Multiple pack size from 10 doses

19	Price to end user	Not more than $0.50/dose	$0.20/dose at end user
20	Storage condition and shelf-life as packaged for sale	12 months at 4-8° C	24 months 4-8° C and/or 48 hours at 30° C
21	In-use stability	1 hour	24 hours

Overall conclusion for improved Bovine anaplasmosis control through vaccination

- It is clear that bovine anaplasmosis is an economically important TBD in all the African and Asian countries targeted in the present report. In East Africa East Coast Fever (ECF) has such prominence as an important disease that other TBDs are overlooked despite their high prevalence. The longitudinal study described earlier, conducted in Kenya on a cohort of calves followed over a 51 weeks period has shown a high rate of *A. marginale* infection, demonstrating the importance of the disease in this East African country where more focus is put on ECF. Similarly, in South and South East Asia, bovine anaplasmosis has a high prevalence, being classified in country like India among the top livestock diseases.
- Despite this, only South Africa has a vaccine which is used regularly. All other countries rely on chemotherapy, with its limitation and long term consequences.
- Vaccination would still be the best way to control the disease, and should be expanded in other affected countries, especially on the African continent. One of the reasons is the fact that livestock keepers end up turning toward antibiotics, most of which are fake or substandard in many countries.
- The current live Theiler *A. centrale* has serious limitations that would prevent its use in more countries.
- With limited research and work toward the development of a vaccine over the past few years, by lack of funds, a new generation vaccine may be a long way away. There may be a possibility of evaluating in target animal the candidates selected by the Washington State University group. These candidate vaccines are likely to lead to a potentially very efficacious and safe vaccine.
- The “quick win” would be the use of the newly established cell culture system for *A.centrale* vaccine strain developed at Pirbright, which could allow the production of a less risky cell-based vaccine. Additionally, work on lyophilisation of the vaccine could be pursued. A cell-based lyophilised bovine anaplasmosis vaccine would then have a major impact on in the control of bovine anaplasmosis.

References

[1] Alfredo AA, Jonsson NN, Finch TM, Neves L, Molloy JB, Jorgensen WK. Serological survey of Babesia bovis and Anaplasma marginale in cattle in Tete Province, Mozambique. Trop Anim Health Prod. 2005 Feb;37(2):121-31.

[2] Aubry P and Geale DW (2011): A Review of Bovine Anaplasmosis. Transboundary and Emerging Diseases. 58 (2011) 1–30

[3] Bastos CV, Passos LM, Facury-Filho EJ, Rabelo EM, Fuente Jde L, Ribeiro MF: Protection in the absence of exclusion between two Brazilian isolates of Anaplasma marginale in experimentally infected calves. Vet J. 2010 Dec;186 (3):374-8. doi: 10.1016/j.tvjl.2009.09.013. Epub 2009 Oct 17.

[4] Belal SMSH, Mahmud MAA and Ferdous MJ, 2014. Prevalence of anaplasmosis in cattle in Sirajganj district of Bangladesh. Res. Agric., Livest. Fish. 1(1): 97-10

[5] Bell-Sakyia L., Palomara A M, Bradford E L, Shkap V: Propagation of the Israeli vaccine strain of Anaplasma centrale in tick cell lines; Veterinary Microbiology 179 (2015) 270–276

[6] Bock RE, de Vos AJ, Kingston TG, McLellan DJ : Effect of breed of cattle on innate resistance to infection with Babesia bovis, B bigemina and Anaplasma marginale; Aust Vet J. 1997 May;75(5):337-40.

[7] DE ANDRADE G M, MACHADO R Z, VIDOTTO M C & VIDOTT O; Immunization of Bovines Using a DNA Vaccine (pcDNA3.1/MSP1b) Prepared from the Jaboticabal Strain of Anaplasma marginale; Annals of the New York Academy of Sciences; v. 1026, n. 1, p. 257-266, 2004.

[8] de la Fuente J, Kocan KM, Blouin EF, Zivkovic Z, Naranjo V, Almazán C, Esteves E, Jongejan F, Daffre S, Mangold AJ. Functional genomics and evolution of tick-Anaplasma interactions and vaccine development Vet Parasitol. 2010 Feb 10;167 (2-4):175-86. doi: 10.1016/j.vetpar.2009.09.019. Epub 2009 Sep 20.

[9] de la Fuente J, Kocan KM, Garcia-Garcia JC, Blouin EF, Claypool PL, Saliki JT. Vaccination of cattle with Anaplasma marginale derived from tick cell culture and bovine erythrocytes followed by challenge-exposure with infected ticks. Vet Microbiol. 2002 Oct 22;89(2-3):239-251

[10] Dennis, R. A.; O'Hara, P. J.; Young, M. F.; Dorris, K. D. Neonatal immunohemolytic anemia and icterus of calves. Journal of the American Veterinary Medical Association 1970 Vol. 156 pp. 1861-1869

[11] Ghosh S & Nagar G; Problem of ticks and tick-borne diseases in India with special emphasis on progress in tick control research: A review; J Vector Borne Dis 51, December 2014, pp. 259–270

[12] Hammac GK, Ku PS, Galletti MF, Noh SM, Scoles GA, Palmer GH, Brayton KA: Protective immunity induced by immunization with a live, cultured Anaplasma marginale strain. Vaccine. 2013 Aug 2;31(35):3617-22. doi: 10.1016/j.vaccine.2013.04.069. Epub 2013 May 9

[13] Hart LT, Luther DG, Todd WJ: Anaplasma marginale antigen, antigen compositions, vaccine and process for the production of said antigen, antigen compositions and vaccine; US patent; 1990 **https://www.google.com/patents/US4956278?dq=ininventor:%22Lewis+T.+Hart%22&hl=en&sa=X&ved=0ahUKEwjgy4DdzerJAhWDcRQKHS0jBqsQ6AEIHTAA**

[14] INDONESIA OIE REPORT FOR 2005 Additional information received from Dr Mathur Riady, Director General of Livestock Services, Ministry of Agriculture, Jakarta **http://www.oie.int/wahis_2/data/reports/uploaded_annual_pdfs/IDN_2005_L1_UploadedAnnualReport.pdf**

[15] Jongejan, F.; Perry, B. D.; Moorhouse, P. D. S.; Musisi, F. L.; Pegram, R. G.; Snacken, M; Epidemiology of bovine babesiosis and anaplasmosis in Zambia; Tropical Animal Health and Production 1988 20 4 234-242

[16] Kiara H., Jennings A., De C. Bronsvoort B. M., Handel I. G., Mwangi S. T., M. Mbole-Kariuki, I. Conradie Van Wyk, E. J. Poole, O. Hanotte, J. A. W. Coetzer, M. E. J. Woolhouse And P. G. Toye (2014). A longitudinal assessment of the serological response to *Theileria parva* and other tick-borne parasites from birth to one year in a cohort of indigenous calves in western Kenya; Parasitology, 141, pp 1289-1298. doi:10.1017/S003118201400050X.

[17] Kivaria FM (2006) ; Estimated direct economic costs associated with tick-borne diseases on cattle in Tanzania. Trop Anim Health Prod. 2006 May;38(4):291-9.

[18] Kocan K M., Blouin E F. & Barbet A F.: Anaplasmosis Control: Past, Present, and Future; Annals of the New York Academy of Sciences; Volume 916, Tropical Veterinary Diseases: Control And Prevention In The Context Of The New World Order pages 501–509, December 2000 **http://onlinelibrary.wiley.com/doi/10.1111/j.1749-6632.2000.tb05329.x/full**

[19] Kocan K.M., de la Fuente J. & Cabezas-Cruz A. (2015): The genus Anaplasma: new challenges after reclassification; Rev. Sci; Tech. Off. Int. Epiz., 2015, 34(2), 577-586

[20] Kocan KM, de la Fuente J, Blouin ED, Coetzee JF, Ewing SA (2010). The natural history of Anaplasma marginale. Vet. Parasitol. 167: 95-107.

[21] Kocan KM, de la Fuente J, Guglielmone AA, Meléndez RD (2003): Antigens and alternatives for control of Anaplasma marginale infection in cattle. Clin Microbiol Rev. 2003 Oct;16(4):698-712

[22] Kocan, K. M., De La Fuente, J., and Blouin, E. F. (2008a). Advances toward understanding the molecular biology of the Anaplasma-tick interface. Front. Biosci. 13, 7032–7045. doi: 10.2741/3208

[23] Mikael Palmfjord (2015): Cross-sectional study of bovine anaplasmosis in South-western Uganda; The impact of wildlife-livestock interface; Department of Biomedical Sciences and Veterinary Public Health; Faculty of Veterinary Medicine and Animal Science; Swedish University of Agricultural Sciences

[24] Minjauw B, Mc Leod A (2003) Tick-borne diseases and poverty. The impact of ticks and tick-borne diseases on the livelihood of small scale and marginal livestock owners in India and eastern and southern Africa. Research report, DFID Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh, UK

[25] Mtshali MS1, de la Fuente J, Ruybal P, Kocan KM, Vicente J, Mbati PA, Shkap V, Blouin EF, Mohale NE, Moloi TP, Spickett AM, Latif AA. Prevalence and genetic diversity of Anaplasma marginale strains in cattle in South Africa. Zoonoses Public Health. 2007;54(1):23-30.

[26] Mutshembele AM1, Cabezas-Cruz A2, Mtshali MS1, Thekisoe OM3, Galindo RC4, de la Fuente J.: Epidemiology and evolution of the genetic variability of Anaplasma marginale in South Africa. Ticks Tick Borne Dis. 2014 Oct;5(6):624-31. doi: 10.1016/j.ttbdis.2014.04.011. Epub 2014 Jul 8.

[27] NGERANWA J.J.N., SHOMPOLE S.P., VENTER E.H., WAMBUGU A., CRAFFORD J.E. and PENZHORN B.L.: Detection of Anaplasma antibodies in wildlife and domestic species in wildlife-livestock interface areas of Kenya by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay; Onderstepoort Journal of Veterinary Research, 75:199–205 (2008)

[28] Nielsen, K., W. L. Yu, L. Kelly, R. Bermudez, T. Renteria, A. Dajer, E. Gutierrez, J. Williams, J. Algire, and S. T. de Eschaide, 2008: Development of a lateral flow assay for rapid detection of bovine antibody to *Anaplasma marginale*. J. Immunoassay Immunochem. 29, 10–18.

[29] Njiiri N.E., B.M. Bronsvoort, N.E. Collins, N.E. Collins, H.C. Steyn, M. Troskie, I. Vorster, S.M. Thumbi, K.P. Sibeko, A. Jennings, I.C. van Vyk, M. Mbole-Kariuki, H. Kiara, E.J. Poole, H. Haotte, K. Coetzer, M.C. Oosthuizen, M. Woolhouse, P. Toye: The Epidemiology of tick-borne haemoparasasites as determined by reverse line blot hybridization assay in an intensively studied cohort of calves in Western Kenya; Vet. Parasitol., 210 (2015), pp. 69–76 **http://dx.doi.org/10.1016/vetpar.2015.02.020**

[30] Noh SM, Brayton KA, Brown WC, Norimine J, Munske GR, Davitt CM & Palmer GH (2008) Composition of the surface proteome of Anaplasma marginale and its role in protective immunity induced by outer membrane immunization. Infect Immun 76: 2219–2226

[31] Nshimiyimana Juvenal and Mutandwa Edward: Seasonal Dynamics and Distribution of Ticks in Rwanda: Implications for Tick Control Strategy in Rwanda; International Journal of Animal and Veterinary Advances 2(1): 21-25, 2010; **http://maxwellsci.com/print/ijava/v2-21-25.pdf**

[32] PALMER G.H.; Sir Arnold Theiler and the discovery of anaplasmosis: A centennial perspective; Onderstepoort Journal of Veterinary Research, 76:75–79 (2009)

[33] Palmer GH, McElwain TF: Molecular basis for vaccine development against anaplasmosis and babesiosis. Vet Parasitol; 1995 Mar;57(1-3):233-53.

[34] Palmer GH1, Rurangirwa FR, Kocan KM, Brown WC Molecular basis for vaccine development against the ehrlichial pathogen Anaplasma marginale. Parasitol Today. 1999 Jul;15(7):281-6.

[35] Potgieter F T and Stoltsz W H. Bovine Anaplasmosis. In: Coetzer JAW &Tustin RC (eds). Infectious diseases of livestock; 2nd ed. South Africa: Oxford, Oxford University Press, 2004; 1037–1070.

[36] Potgieter F T and Van Rensburg L: Tick Transmission Of Anaplasma Centrale Onderstepoort J. Vet. Res., 54,5-7 (1987)

[37] Rachel Kenneil (2015): The immune response to live vaccines against bovine anaplasmosis; PhD thesis; faculté des Sciences; Institut de Biologie; Université de Neuchâtel, Switzerland

[38] Sebele T, Zewedu F and Getachew AG: A study of the prevalence of hemoparasites of ruminants in and around Debre-Zeit, Central Ethiopia; African Journal of Parasitology Research Vol. 2 (3), pp. 066-071, March, 2015.

[39] Simuunza, Martin Chitolongo (2009) Differential diagnosis of tick-borne diseases and population genetic analysis of Babesia bovis and Babesia bigemina. PhD thesis 2009; University of Glasgow; **http://theses.gla.ac.uk/1240/1/2009simuunzaphd.pdf**

[40] Swai E. S., Karimuribo E. D., Ogden N. H., French N. P., Fitzpatrick J. L., M. J. Bryant , D. M. Kambarage. Seroprevalence Estimation and Risk Factors for *A. marginale* on Smallholder Dairy Farms in Tanzania; Tropical Animal Health and Production; November 2005, Volume 37, Issue 8, pp 599-610

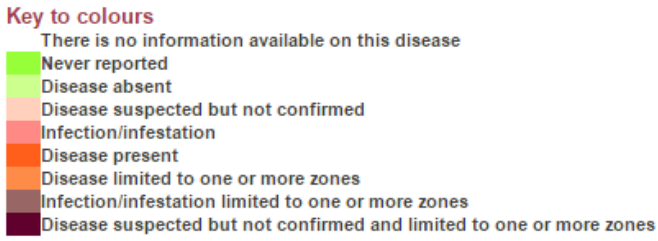
[41] Tefi I K, Satrija F and Cahyaningsih U: Study the Existence of Blood Parasites (Anaplasma, Babesia, Theileria) and Physiological Profiles of Australian Imported Feeder Cattle; Acta Parasitologica Globalis 6 (1): 55-59, 2015

[42] Texas Agric. extension: **http://animalscience.tamu.edu/wp-content/uploads/sites/14/2012/04/beef-anaplasmosis.pdf**

[43] World Organization for Animal Health: OIE Terrestrial Manual. Manual of Diagnostic tests and vaccines for terrerstrial animals 2015. Accessed on line. **http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/**.

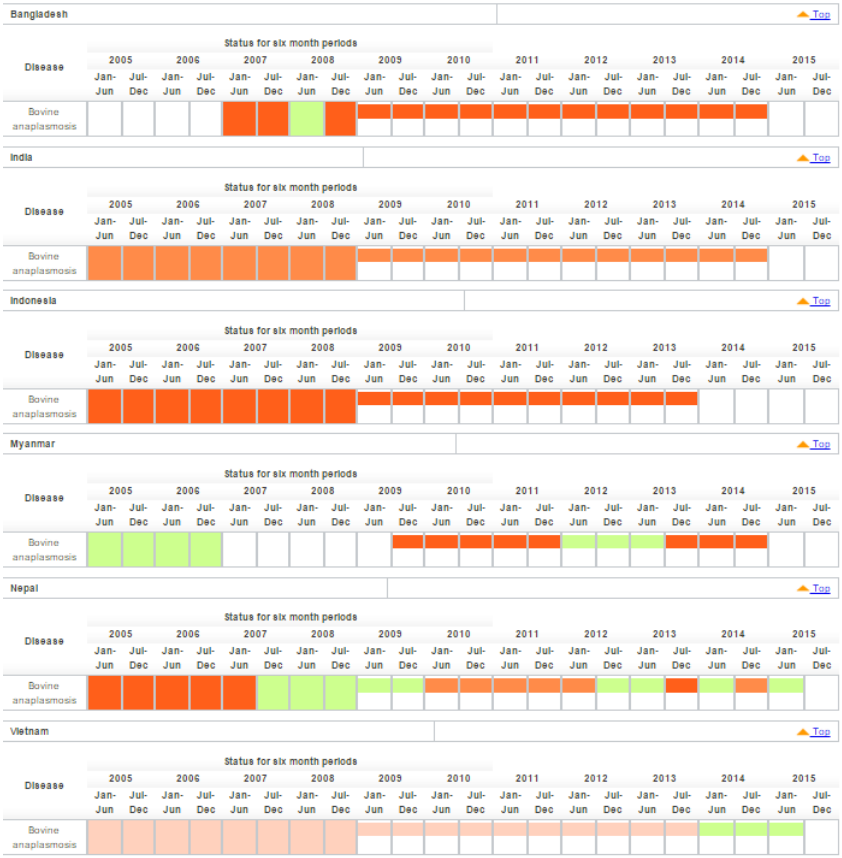
ANNEX 1: Additional data on disease presence and incidence

Reports to OIE on Bovine anaplasmosis:

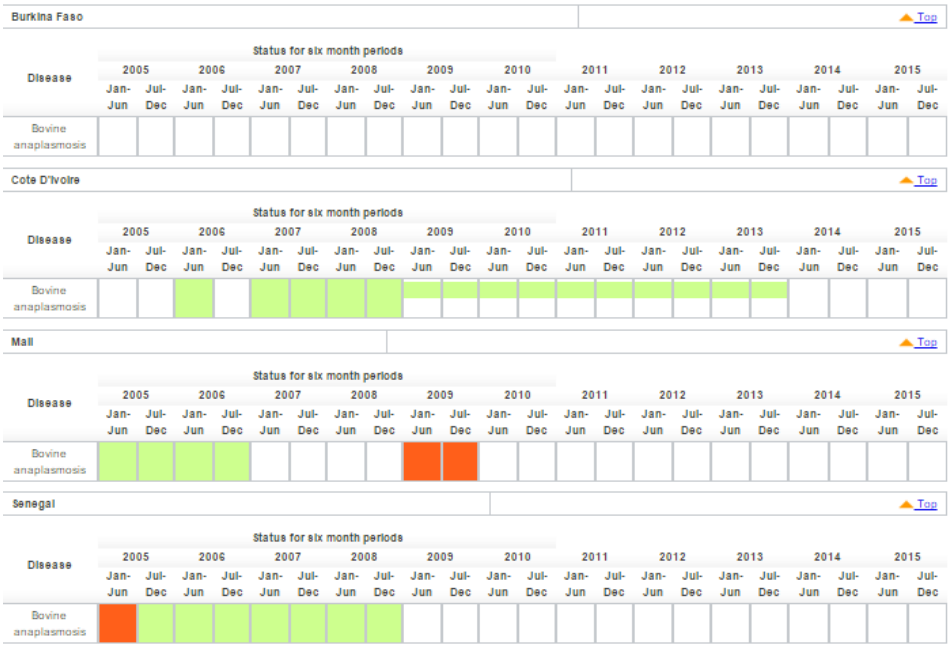


When different animal health statuses between domestic and wild animal population are provided, the box is split in two: the upper part for domestic animals, and the lower part for wild animals.

Bovine anaplasmosis in Asia: Bangladesh, India, Indonesia, Myanmar, Nepal and Vietnam



Bovine anaplasmosis in Western Africa: Burkina Faso, Ivory Coast, Mali and Senegal



Bovine anaplasmosis in Eastern Africa: Ethiopia, Kenya, Rwanda, Tanzania and Uganda

Bovine anaplasmosis in Southern Africa: Madagascar, Malawi, Mozambique, South Africa and Zambia